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Responses of mosquitoes and German cockroaches to ultrasound emitted from a random ultrasonic generating device

Aqeel Ahmad[1]*, Bhadriraju Subramanyam[2] & Ludek Zurek[1]

[1]*Department of Entomology, Kansas State University, Manhattan, KS 66506, USA,* [2]*Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506, USA*

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Abstract

The repellency of ultrasound to females of two species of mosquitoes, *Anopheles quadrimaculatus* Say and *Anopheles gambiae* Giles (Diptera: Culicidae), and male and female German cockroaches, *Blattella germanica* (L.) (Blattodea: Blattellidae), was evaluated under laboratory conditions using a random ultrasonic device developed at Kansas State University. This device produces ultrasound in the 20–100 kHz frequency range and random sound patterns at any frequency range. Under the particular settings described in the paper, this ultrasonic device produced sound pressure levels from 91 to 101, 91 to 102, and 90 to 100 dB at the top, bottom, and side panels of the test chamber, respectively (0 dB = 20 micropascals). Sound pressure levels recorded at the center of the top, bottom, and side panels were higher than those recorded at the panel edges. Ultrasound from the random ultrasonic device failed to repel mosquitoes and German cockroaches at the different frequency ranges evaluated. Our results confirm previous findings with commercial devices producing constant sound patterns that ultrasound in general is not a promising tool for repelling mosquitoes and cockroaches.

Introduction

Several commercial ultrasonic devices evaluated in either laboratory or field tests against various species of mosquitoes (Diptera: Culicidae) (Kutz, 1974; Garcia et al., 1976; Singleton, 1977; Belton, 1981; Lewis et al., 1982; Foster & Lutes, 1985; Cabrini & Andrade, 2006) and German cockroaches (Blattodea: Blattellidae) (Ballard & Gold, 1983; Gold et al., 1984; Schreck et al., 1984; Koehler et al., 1986; Huang & Subramanyam, 2006) failed to effectively repel the pests. The devices used in most of these studies had frequencies of only 2–5 kHz, with harmonics extending into the ultrasounic range. Sound pressure generated by these devices varied from 68 to 84 dB at 1 cm distance from the source. Gold et al. (1984) found that the sound output of commercially available ultrasonic devices was less than what the manufacturers claimed. To date, frequencies in the range of 2–60 kHz (mosquitoes) and 20–60 kHz (German cockroach) generated by different commercial ultrasonic devices were evaluated, and none showed a clear repellency effect. The degree of repellency may depend on the frequency/intensity of the ultrasound. Foster & Lutes (1985) suggested that higher frequencies or increased sound pressure may have a deterrent effect.

All current commercial ultrasonic devices produce constant sound patterns and insects may become habituated when exposed to devices that produce these constant sound patterns. Therefore, it was desirable to develop an ultrasound device that produced random sound patterns over time. One such device was developed in 2001 by the Electronics Design Laboratory at Kansas State University (KSU). In this study, we describe and characterize the sound patterns of the KSU device, and determine the efficacy of ultrasound as a repellent against two species of mosquitoes and German cockroaches.

*Correspondence: Aqeel Ahmad, Department of Entomology, Kansas State University, 123 Waters Hall, Manhattan, KS 66506-4004, USA. E-mail: aahmad@ksu.edu

Materials and methods

Test species

A laboratory colony of two mosquito species (*Anopheles quadrimaculatus* Say and *Anopheles gambiae* Giles) was established and maintained at 28 °C, 70% r.h., and an

L14:D10 cycle in the Department of Entomology, Kansas State University, Manhattan, KS, USA. Larvae were maintained on 2% larval food (a mixture of baker's yeast and fry bites® fish food at a 1:2 ratio; HBH Pet Products, Springville, UT, USA). Pupae were gently harvested within 24 h of pupation and placed in clean water in a Styrofoam bowl. The Styrofoam bowls with pupae were transferred to nylon gauze stock cages (30 × 30 × 30 cm, BugDorm-1, MegaView Science Education Services Co., Ltd, Taiwan). Adults emerging from pupae were provided with a 10% sucrose solution. One week after adult emergence, adult males were aspirated from the stock cages. Unfed female mosquitoes between 7 and 10 days old were used in tests.

The German cockroach, *Blattella germanica* (L.), was obtained from the Department of Entomology, North Carolina State University, Raleigh, NC, USA. Cockroaches were reared on Friskies® cat food (Nestle Purina Petcare Co., St. Louis, MO, USA) in plastic containers (40 × 26 × 13.5 cm, Rubbermaid Inc., Wooster, OH, USA). Water was provided by placing a water-soaked cotton swab in a plastic cup. The cockroach culture was held in the laboratory at 28 °C, 70% r.h., and an L14:D10 cycle.

Voucher specimens of *A. quadrimaculatus*, *A. gambiae*, and *B. germanica* were submitted to the Insect and Prairie Arthropod Research Museum at Kansas State University (Voucher Specimen no. 187).

Ultrasound device and sound measurements

The KSU ultrasonic device uses a computer, arbitrary waveform generator and custom electronics to generate ultrasonic pulses in the 20–100 kHz frequency range. The computer randomly chooses the pulse length, frequency, and quiet time between pulses across the entire frequency range (Wichmann & Hill, 1982; Huang & Subramanyam, 2004). The computer then directs the arbitrary waveform generator to construct the pulse. The output of the arbitrary waveform generator is then amplified with custom electronics to the requirements of the electrostatic transducer that ultimately produces the desired ultrasound. One device can drive two ultrasonic transducers simultaneously.

Sound pressure levels (SPLs) were measured using a 0.64-cm Bruel and Kjaer (B and K) type 4939 condenser microphone, a B and K type 2670 preamplifier, and a B and K NEXUS® conditioning amplifier (Bruel and Kjaer, North America, Norcross, GA, USA). Measurements were made at a distance of 0, 50, and 100 cm from the device transducers to determine the sound attenuation over distance within the frequency range containing the peak SPL. Measurements were calibrated using a B and K type 4231 sound level calibrator. The sound signal was digitized at 200,000 samples per second using a 12-bit PCMCIA data acquisition card. A laptop computer and a custom software interface were used to display, print, and record data. The custom software displays the digitized waveform, frequency spectrum of the waveform, and the SPL. In the current study, the KSU device was tested at a frequency range of 60–100 kHz (in 5 kHz increments), a sound duration of 50–200 ms (random increments), a quiet time of 50–500 ms (random increments), and an amplitude of 2.25. The SPL measurements were also made at the center and corners of the top, bottom, and side panels of the chamber used in this study.

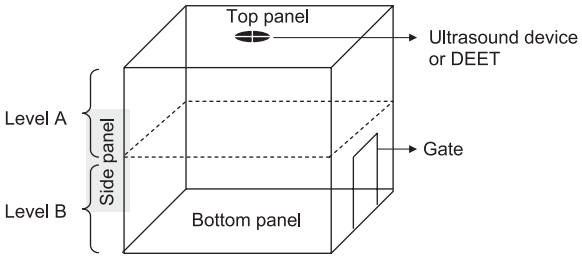

Figure 1 Diagram of plexiglas chambers, used in experiments with mosquitoes, showing top, bottom, and side panels.

Evaluation of ultrasound against mosquitoes

The KSU ultrasound device was tested to evaluate the efficacy using a frequency range of 60–70 kHz (*A. quadrimaculatus*), 70–80 kHz (*A. quadrimaculatus* and *A. gambiae*), 80–90 kHz (*A. quadrimaculatus* and *A. gambiae*), and 90–100 kHz (*A. quadrimaculatus*) while keeping other parameters as described above. Three plexiglas chambers (1.2 × 1.2 × 1.2 m) (Figure 1) were randomly allotted to three treatments (control, ultrasound, and DEET). In one chamber, two transducers of the KSU ultrasound device were mounted in the center of the top panel. A commercial repellent, OFF! Deep Woods (SC Johnson, Racine, WI, USA) containing 30% DEET (*N*,*N*-diethyl-*m*-toluamide), was used as positive control by spraying it on a piece (30.48 × 30.48 cm) of black cotton cloth. The sprayed cotton cloth was hung in the top center of one of the chambers. The third chamber without the transducers and DEET served as the control treatment. A total of four tests (replicates) were conducted for each treatment. Each of four side panels of each chamber was divided into two levels, referred to as 'level A' (the section close to the active transducer/DEET sprayed cloth) and 'level B' (the section far from the active transducer/DEET sprayed cloth). Female mosquitoes (~200) were released from stock cages into each chamber and allowed to acclimate for 30 min. Mosquitoes were counted on the top and bottom panel and each level of side panels to assess the distribution before any treatment exposure. The KSU ultrasonic device was turned on for 30 min. After 30 min of exposure, the

distribution of mosquitoes was recorded by counting the number of mosquitoes on the top and bottom panel and each level of the side panels in each treatment. A new group of mosquitoes was used for each test (replicate). After each test, all insects in the test chambers were vacuumed and test chambers were washed with non-fragrance soap and water and rinsed between each test. A microprocessor-based sensor (HOBO unit, Onset Computer Corporation, Bourne, MA, USA) was installed in the center of the bottom panel of each chamber to record temperature and relative humidity. Temperature and relative humidity during all tests was 27–29 °C and 60–80%, respectively.

Data on the number of mosquitoes on the top and bottom panel and each level of the side panels across all replications in each treatment were analyzed, and expressed as a percentage by dividing the total number of mosquitoes at a particular level (Level A or B) of the side, top, or bottom panel by the total number of mosquitoes in that chamber.

Evaluation of ultrasound against German cockroaches

Two different types of experiments were conducted. In the first experiment, groups of unsexed nymphs and adults German cockroaches of mixed ages (20% nymphs and 80% adults) were held in different stock cages and provided with water-soaked cotton balls. Paired plexiglas chambers ($1.2 \times 1.2 \times 1.2$ m) were used for each test. A 91-cm-long square conduit, measuring 7.6 cm^2, connected the two chambers at the bottom front corner. Rectangular plexiglas gates near the conduit junctions could be opened manually to allow insect movement between the chambers (Huang & Subramanyam, 2004, 2006). The KSU ultrasound device was mounted in the top center of each chamber and faced directly toward the center of the chamber's floors. In the control tests, the KSU ultrasound device was mounted similarly, but was not activated. Prior to insect releases, two transparent plastic Petri dishes (100 × 15 mm, Fisherbrand, Fisher, Pittsburgh, PA, USA), one containing 50 g of Friskies® cat food and the another containing cotton balls saturated with distilled water, were placed at the center of the floor in each chamber. HOBO data logging units were installed in each chamber to record temperature and humidity levels. Temperature and relative humidity during all tests was 25–27 °C and 60–78%, respectively.

One hundred German cockroaches were released from stock cultures (cages) into each chamber and allowed to acclimate for 24 h (day 1). The plexiglas gates at the junctions were closed during this time period, and then they were opened to allow insects to move freely between the chambers for another 24 h (day 2). After this initial 48 h, the KSU ultrasonic device in one of the paired chamber (randomly selected) was turned on (active) for 3 days (days 3–5) and then turned off (first 3-day test period). The ultrasonic device in the other chamber was then turned on for an additional 3 days (days 6–8) (second 3-day test period). This 8-day test (48 h + 6 days) constituted a single replication. Tests with the KSU ultrasound device and the control were replicated three times. The chambers were covered with black plastic sheets to exclude light during tests. The plastic sheets were removed and the gates were closed temporarily to facilitate counting.

The number of cockroaches in each chamber was visually counted daily and the number of dead cockroaches in the chambers was also recorded at the end of each test. A new group of cockroaches was used for each replicate. After each test, all insects in the test chambers were vacuumed and test chambers were washed with non-fragrance soap and water and rinsed between each test.

In the second experiment, a pair of plexiglas chambers ($1.2 \times 1.2 \times 1.2$ m) with a kitchen cabinet ($30.5 \times 101.6 \times 30.5$ cm) was used for each test. The kitchen cabinet stocked with different food items including cereals, chips, bread, and nuts was placed in the center of each chamber. The KSU ultrasound device was mounted in the top center of each chamber. Another KSU ultrasound device was mounted inside the kitchen cabinet and faced directly toward the food items. Tests with KSU ultrasound device and the control were replicated three times and the chamber treatments (with or without ultrasound) were assigned at random. In each replication, a total of 150 unsexed nymphs and adults of mixed ages (20% nymphs and 80% adults) were released from stock cages into each chamber and allowed to acclimate for 24 h (day 1). The KSU ultrasonic device in one of the chambers was turned on for 3 days and then turned off (first 3-day test period). Then the ultrasonic device inside the kitchen cabinet in the same chamber was then turned on for 3 days (second 3-day test period). Another chamber and kitchen cabinet was used as control (without any ultrasound). This 7-day test (24 h + 6 days) was replicated three times. The chambers were covered with black plastic sheets to exclude light during tests. The plastic sheets were removed temporarily to facilitate counting.

The number of cockroaches in each chamber was visually counted daily to assess numbers inside the kitchen cabinet. A new group of cockroaches was used for each replicate. After each test, all insects in the test chambers and kitchen cabinets were removed by vacuuming.

Data analysis

Data for percent distribution of mosquitoes were transformed to angular values to normalize heteroscedastic treatment variations (Gomez & Gomez, 1984) and analyzed using analysis of variance (ANOVA). Mean separations were carried out using the least-squares means procedure

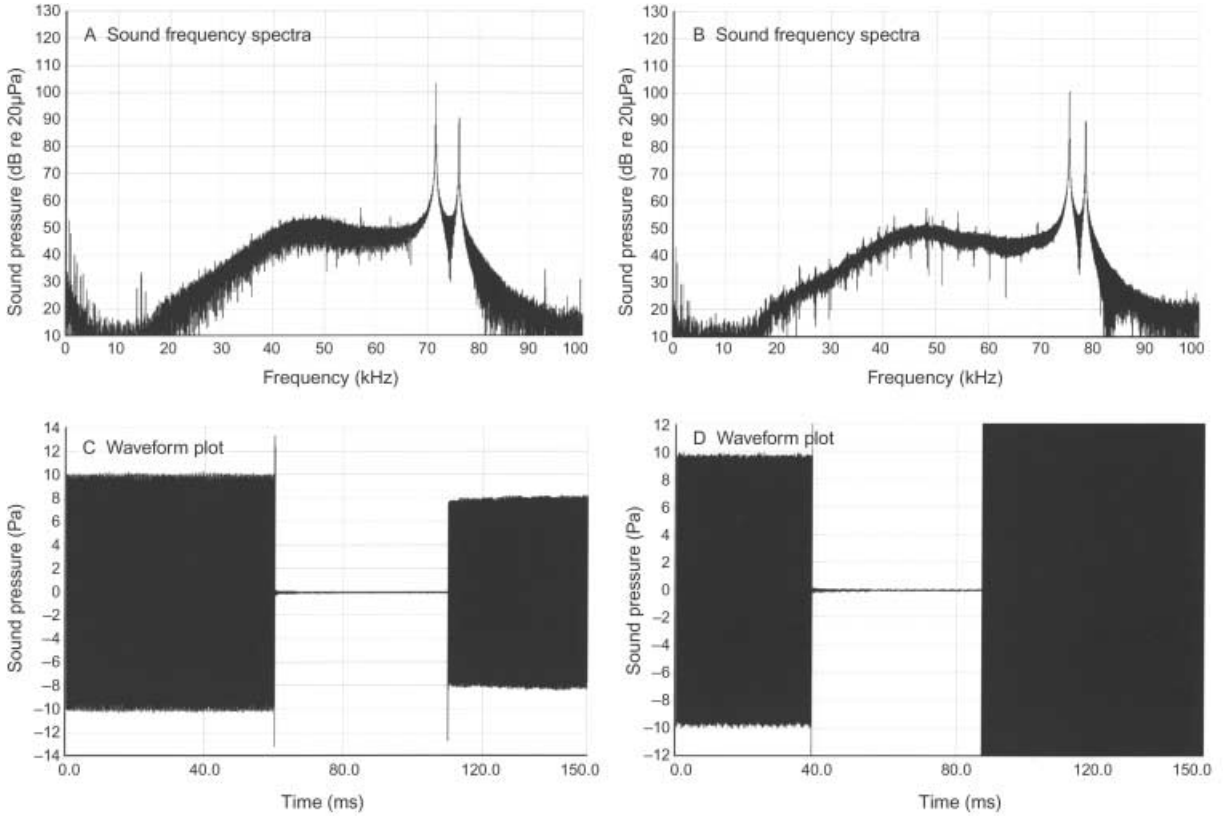

Figure 2 Measurement of sound patterns from Kansas State University (KSU) ultrasonic device at the 70–80 KHz frequency range at 50 cm from the source.

(P value = 0.05) of the general linear model (GLM) (SAS Institute, 2003). Although all tests of significance were based on the transformed data, the untransformed percent distribution is reported.

Data on the daily number of cockroaches in the paired chambers (first experiment) were analyzed by paired t-tests (SAS Institute, 2003), to determine difference between chambers with the KSU device status (active and inactive) at a specific date and between the chambers in the control tests. Data on the daily number of cockroaches in the second experiment were analyzed by two sample t-tests (SAS Institute, 2003), to compare the distribution inside and outside the kitchen cabinets at a specific date between control chambers and chambers with the KSU device.

Results

Sound measurements and characterization

Sound characterization of the KSU ultrasound device was carried out at four different frequency ranges, 60–70, 70–80, 80–90, and 90–100 kHz. The SPLs at each tested frequency range decreased with increasing distance from the transducer (about 10 dB SPL decrease per doubling of distance from the sound source).

The change in sound frequencies and waveform plots over time indicated that these changes were random. For example, the sound patterns in the 70–80 kHz frequency range shown in Figure 2 had peak frequencies at 71.5 kHz and 76.5 kHz in one output (Figure 2A) and peak frequencies at 76.0 kHz and 78.5 kHz in the next output (Figure 2B). The waveform plot (Figure 2C,D) showed the sound cycle duration to be 150 ms. In each sound cycle, there were two groups of pulses and each group was characterized by multiple pulses. Outputs with other frequency ranges (60–70, 80–90, and 90–100 kHz) showed similar variation (data not shown).

Sound pressure level distributions within a chamber were slightly different among top panel, bottom, and side panels of the chamber. SPLs ranged from 91 to 101, 91 to 102, and 90 to 100 dB at the top, bottom, and side panels, respectively. SPLs recorded at the central area of the top panel, bottom, and side panels were higher than those recorded from the border area of the panels.

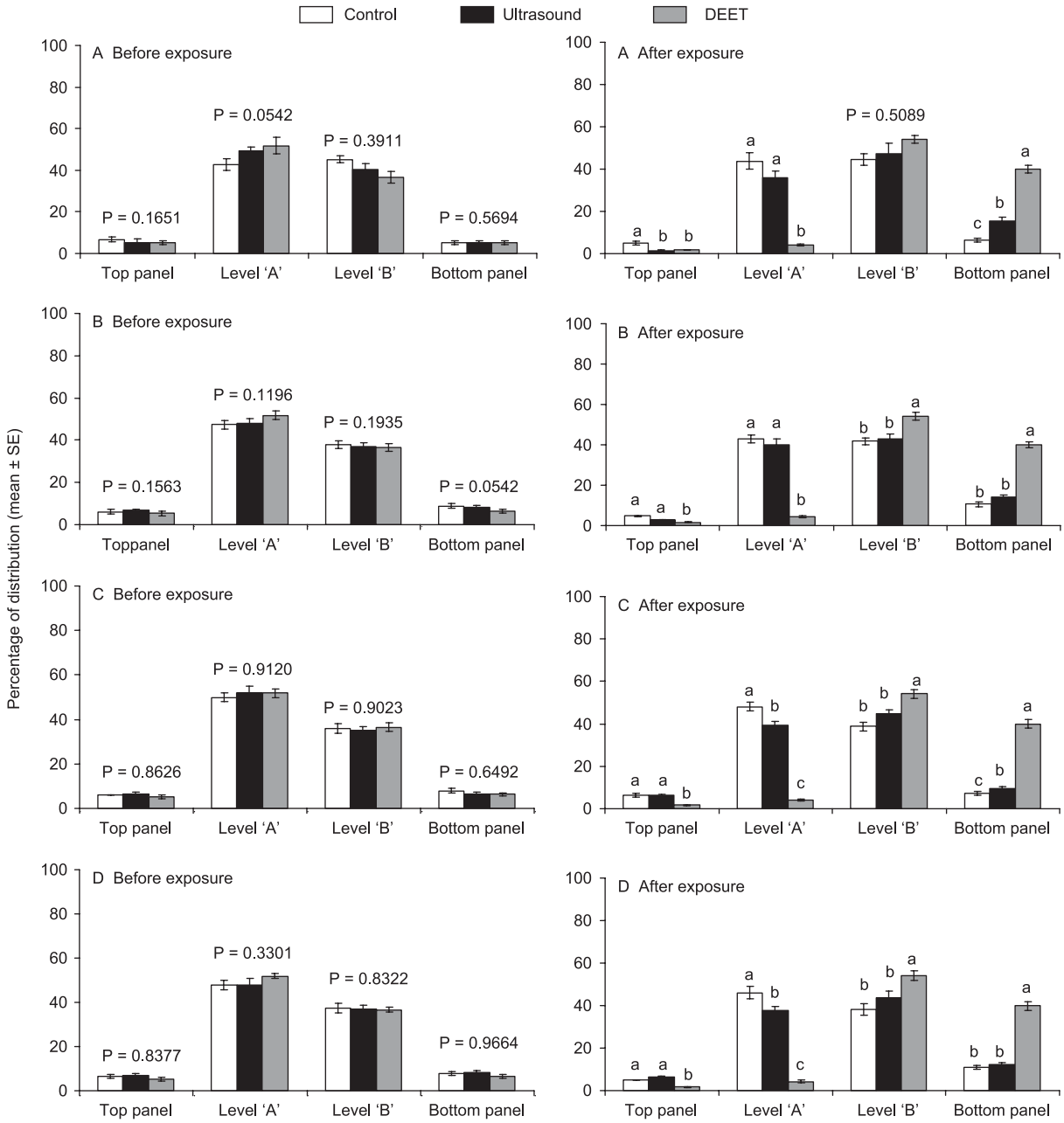

Figure 3 Distribution of *Anopheles quadrimaculatus*, expressed as percentage of total insects, within a chamber in the absence or presence of ultrasound and DEET. Treatment means within a panel per level topped with different letters are significantly different ($P<0.05$; LS-mean test) (A) before and after exposure to ultrasound and DEET: 60–70 kHz, (B) before and after exposure to ultrasound and DEET: 70–80 kHz, (C) before and after exposure to ultrasound and DEET: 80–90 kHz, and (D) before and after exposure to ultrasound and DEET: 90–100 kHz.

Evaluation of ultrasound against mosquitoes

The KSU device was tested against *A. quadrimaculatus* at four different frequency ranges, 60–70, 70–80, 80–90, and 90–100 kHz. No significant differences in overall distribution of *A. quadrimaculatus* were observed among treatments before the treatment exposure (Figure 3A, before exposure; 3B, before exposure; 3C, before exposure; 3D, before exposure), suggesting that *A. quadrimaculatus* was evenly distributed at the top panel, level A and level B of all side panels, and bottom panel.

Figure 3A (after exposure) shows the results of overall distribution of *A. quadrimaculatus* in a chamber with ultrasound exposure at 60–70 kHz frequency range compared with other treatments, i.e., chamber with DEET application and control chamber. DEET application provided significant reduction of *A. quadrimaculatus* at level A of all side panels ($F_{2,9} = 49.23$, $P<0.0001$) compared to other treatments. However, the chambers with ultrasound and control chamber did not differ significantly in overall distribution of *A. quadrimaculatus* at level A of all side panels. No significant difference in percentage of *A. quadrimaculatus* occurred among treatments at level B of all side panels ($F_{2,9} = 0.73$, $P = 0.5089$). Significant differences occurred in overall distribution of *A. quadrimaculatus* at the top panel ($F_{2,9} = 3.11$, $P = 0.0474$) and bottom panel ($F_{2,9} = 33.3$, $P<0.0001$) in the presence of DEET or ultrasound compared to the control chamber. However, a significantly higher percentage of *A. quadrimaculatus* was found on the bottom panel of the chamber with DEET compared to the chamber with ultrasound.

Figure 3B (after exposure) shows the results on overall distribution of *A. quadrimaculatus* in chamber with an ultrasound exposure at 70–80 kHz frequency range compared to other treatments. DEET application provided a significant reduction of *A. quadrimaculatus* at the top panel ($F_{2,9} = 32.56$, $P = 0.0401$) and level A of all side panels ($F_{2,9} = 91.44$, $P<0.0001$) compared to other treatments. Likewise, a significantly greater percentage of *A. quadrimaculatus* were found at level B of all side panels ($F_{2,9} = 5.83$, $P = 0.0238$) and bottom panel ($F_{2,9} = 33.3$, $P<0.0001$) in the presence of DEET. However, the chambers with ultrasound at 70–80 kHz frequency range and control chamber did not differ in overall distribution of *A. quadrimaculatus* at both levels (A and B) of all side panels, the top panel and the bottom panel.

Results of ultrasound at 80–90 kHz (Figure 3C, after exposure) and 90–100 kHz (Figure 3D, after exposure) frequency ranges were similar to those at 70–80 kHz frequency range. The chamber with ultrasound and control chamber did not differ in the overall distribution of *A. quadrimaculatus* at level B of all side panels, the top panel, and the bottom panel. However, a significantly lower percentage of *A. quadrimaculatus* was found at level A of all side panels in the presence of ultrasound at the 90–100 kHz frequency range compared with the control chamber. DEET application also provided a significant reduction in *A. quadrimaculatus* numbers on level A of all side panels (80–90 kHz: $F_{2,9} = 149.65$, $P<0.0001$; 90–100 kHz: $F_{2,9} = 125.01$, $P<0.0001$), and the top panel (80–90 kHz: $F_{2,9} = 3.24$, $P = 0.0417$; 90–100 kHz: $F_{2,9} = 3.51$, $P = 0.0321$) compared with other treatments. In the presence of DEET, a significantly greater percentage of *A. quadrimaculatus* were found at level B on all side panels (80–90 kHz: $F_{2,9} = 5.95$, $P = 0.0225$; 90–100 kHz: $F_{2,9} = 6.27$, $P = 0.0197$) and on the bottom panel (80–90 kHz: $F_{2,9} = 37.49$, $P<0.0001$; 90–100 kHz: $F = 31.55$, $P<0.0001$) compared with other treatments.

The KSU device was tested against *A. gambiae* at 70–80 kHz and 80–90 kHz frequency ranges. There were no significant differences in overall distribution of *A. gambiae* among treatments before the treatment exposure (Figure 4A, before exposure and 4B, before exposure), suggesting that the distribution was essentially equal at top, bottom, and each level of side panels.

Figure 4A and 4B (after exposure) show the results on overall distribution of *A. gambiae* in chamber with ultrasound exposure at 70–80 kHz and 80–90 kHz frequency ranges compared to other treatments. A significantly lower percentage of *A. gambiae* was found on the top panel and level A of all side panels in the presence of DEET or ultrasound compared to control chamber. However, DEET application also provided a significant reduction in *A. gambiae* numbers on the top panel (70–80 kHz: $F_{2,9} = 10.86$, $P<0.0001$; 80–90 kHz: $F_{2,9} = 12.28$, $P<0.0001$) and level A of all side panels ($F_{2,9} = 124.62$, $P<0.0001$; 80–90 kHz: $F_{2,9} = 227.31$, $P<0.0001$) compared to the chamber with ultrasound at 70–80 kHz (Figure 4A, after exposure) and 80–90 kHz (Figure 4A, after exposure) frequency ranges. In the presence of DEET, a significantly greater percentage of *A. gambiae* was found at level B of all side panels (70–80 kHz: $F_{2,9} = 12.25$, $P = 0.0027$; 80–90 kHz: $F_{2,9} = 15.44$, $P = 0.0012$) and bottom panel (70–80 kHz: $F_{2,9} = 16.97$, $P<0.0001$; 80–90 kHz: $F_{2,9} = 25.36$, $P<0.0001$) compared to other treatments. Significant differences also occurred in the overall distribution of *A. gambiae* at level B of all side panels in the presence of ultrasound compared to the control chamber (70–80 kHz: $F_{2,9} = 12.25$, $P = 0.0027$; 80–90 kHz: $F = 15.44$, $P = 0.0012$).

Evaluation of ultrasound against German cockroaches

In the first experiment, there were no significant differences in the distribution of cockroaches between the control paired chambers throughout the test period (day 2, $P = 0.6257$; day 3, $P = 0.7952$; day 4, $P = 0.1363$; day 5, $P = 0.3440$; day 6, $P = 0.8512$; day 7, $P = 0.7952$; day 8, $P = 0.2816$). In addition, in the tests with the KSU ultrasound device, the number of cockroaches observed on day 2 before the ultrasound device was turned on was also not significantly different between the paired chambers ($P = 0.9261$) (Table 1). These results suggest that the cockroach population was evenly distributed between the paired chamber in the control tests and in the tests with ultrasound device before the ultrasound device was turned on. No mortality of cockroaches was observed in any of the

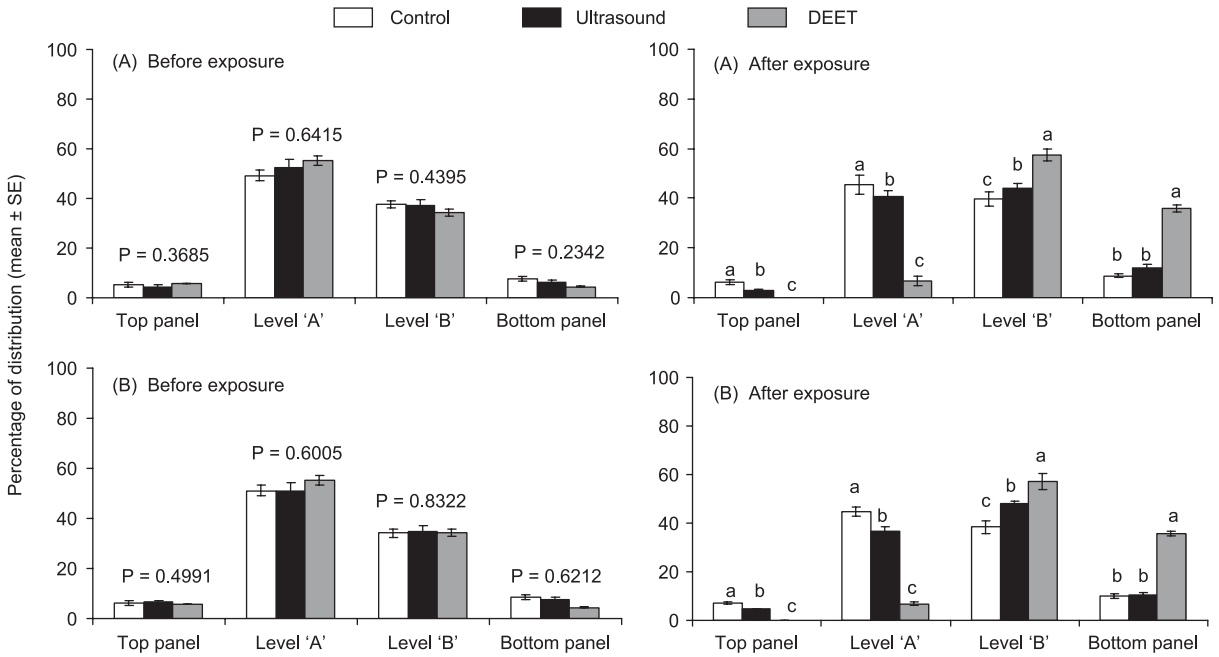

Figure 4 Distribution of *Anopheles gambiae*, expressed as percentage of total insects, within a chamber in the absence or presence of ultrasound and DEET. Treatment means within a panel per level topped with different letters are significantly different (P<0.05; least-squares means test) (A) before and after exposure to ultrasound and DEET (70–80 kHz), and (B) before and after exposure to ultrasound and DEET (80–90 kHz).

tests. The unaccounted cockroaches were found in the conduits connecting the chambers.

In the paired tests with KSU ultrasound device after the device was turned on (active), the number of cockroaches in the chambers with active ultrasound device were lower than that found in the chambers with the inactive device throughout the first 3-day test period (days 3–5). The paired t-tests indicated that these differences in numbers were not statistically significant throughout the first 3-day test period (day 3, P = 0.1164; day 4, P = 0.0650; day 5, P = 0.0617) (Table 1). However, no significant differences in the number of cockroaches were observed in the second 3-day test period (days 6–8) between the paired chambers. Cockroaches did not move from the chamber where the ultrasound device was turned on for the second 3-day test period (day 6, P = 0.3206; day 7, P = 0.8404; day 8, P = 0.5432) (Table 1).

In the second experiment, on the first day before the ultrasonic device was turned on, there was no significant difference in the number of cockroaches outside of kitchen

Table 1 Daily number (mean ± SE) of German cockroaches within paired chambers in the absence and presence of ultrasound

	Control		Ultrasound	
Day	Inactive	Inactive	Active	Inactive
2	90.0 ± 3.0a	96.0 ± 6.0a	93.7 ± 1.8a	94.3 ± 4.8a
First 3-day test				
3	91.5 ± 1.5a	92.0 ± 3.0a	90.3 ± 2.9a	102.0 ± 1.5a
4	80.0 ± 7.0a	99.5 ± 2.5a	83.7 ± 3.2a	105.3 ± 2.9a
5	95.0 ± 2.0a	90.0 ± 1.0a	89.0 ± 2.7a	105.3 ± 2.7a
Second 3-day test				
6	91.5 ± 0.5a	89.0 ± 10.0a	96.7 ± 1.2a	94.7 ± 1.5a
7	94.0 ± 4.0a	91.0 ± 5.0a	95.0 ± 5.0a	97.3 ± 5.2a
8	100.5 ± 2.5a	91.0 ± 2.0a	94.0 ± 3.1a	98.0 ± 3.1a

Means in the paired enclosures at a specific observation followed by the same letter are not significantly different (P>0.05; paired t-test).

Table 2 Daily number (mean ± SE) of German cockroaches outside and inside of kitchen cabinets in the control chamber and the chamber with ultrasound device

	Outside kitchen cabinet		Inside kitchen cabinet	
Days	Control	Ultrasound	Control	Ultrasound
1	132.0 ± 3.5a	136.3 ± 2.9a	18.0 ± 3.5a	13.7 ± 2.9a
First 3-day test[1]				
2	103.7 ± 2.9a	94.3 ± 2.7a	46.3 ± 2.9a	55.7 ± 2.7a
3	69.7 ± 5.0a	65.3 ± 6.1a	80.3 ± 5.0a	84.7 ± 6.1a
4	47.3 ± 7.6a	46.0 ± 16.6a	102.7 ± 7.6a	104.0 ± 16.6a
Second 3-day test[2]				
5	37.5 ± 6.1a	29.5 ± 6.1a	112.5 ± 6.1a	120.5 ± 6.1a
6	27.3 ± 6.2a	26.7 ± 3.4a	122.7 ± 6.2a	123.3 ± 3.4a
7	28.7 ± 7.0a	30.7 ± 7.2a	121.3 ± 7.0a	119.3 ± 7.2a

[1]Ultrasound device active in chamber but inactive inside kitchen cabinet.
[2]Ultrasound device active inside kitchen cabinet but inactive in chamber.
Means within the same row at a specific observation followed by the same letter are not significantly different for the cockroach distribution (P>0.05; two-sample t-test).

cabinets in the control chamber and the chamber with ultrasound. Likewise, the number of cockroaches inside kitchen cabinets with and without ultrasound were not significantly different (P = 0.3921) (Table 2). These results suggest the cockroach population was evenly distributed on the first day before the ultrasonic device was turned on.

Table 2 indicates that the number of cockroaches outside kitchen cabinets consistently decreased in both chambers whether ultrasound was present or not for first 3-day test period. Correspondingly, the number of cockroaches consistently increased inside kitchen cabinets with or without ultrasound of the same chambers in the first 3-day test period. These changes in the number of cockroaches were also observed during the second 3-day test period. However, no significant difference in number of cockroaches was observed outside of kitchen cabinets in the control chambers and the chambers with ultrasound during the first 3-day test period (day 2, P = 0.0793; day 3, P = 0.6120; day 4, P = 0.9454) and for the second 3-day test period (day 5, P = 0.5294; day 6, P = 0.9291; day 7, P = 0.8512). Similarly, no significant difference in number of cockroaches occurred inside of kitchen cabinets with and without ultrasound during the first 3-day test period (day 2, P = 0.0793; day 3, P = 0.6120; day 4, P = 0.9454) and for the second 3-day test period (day 5, P = 0.5294; day 6, P = 0.9291; day 7, P = 0.8512). These results indicated that cockroaches were just as likely to enter kitchen cabinets treated with or without ultrasound (control) cabinets.

Discussion

The purpose of this study was to evaluate the effects of ultrasound against mosquitoes and German cockroaches using a KSU ultrasonic device that produces ultrasound in the 20–100 kHz frequency range and random sound patterns across the entire frequency range.

All tests were conducted in an empty storage room that had no windows. Sound measurements in this room indicated it to be free of any external noise (data not shown). The KSU ultrasound device produced very strong ultrasounds with a random sound pattern over time. The results of our study did not provide evidence that the ultrasound can repel mosquitoes and cockroaches. Furthermore, there is no reason to expect a different effect on these insects if they were exposed to ultrasound devices producing higher frequencies and random sound patterns. The results observed in this study are consistent with those found with commercial ultrasonic devices against several species of mosquitoes (Kutz, 1974; Garcia et al., 1976; Singleton, 1977; Belton, 1981; Lewis et al., 1982; Foster & Lutes, 1985; Cabrini & Andrade, 2006) and German cockroaches (Ballard & Gold, 1983; Gold et al., 1984; Schreck et al., 1984; Koehler et al., 1986; Huang & Subramanyam, 2006). The use of ultrasound in some experiments resulted in statistically significant differences in distribution of mosquitoes within a chamber compared to a chamber without ultrasound. However, the magnitude of these differences in mosquito distribution was significantly lower than that with DEET treatment. Therefore, the practical application of ultrasound against mosquitoes was less than convincing. This could be due to the fact that the antennae of females are less sensitive to sound stimuli than those of males because of fewer sound receptors (McIver, 1985; Michelsen & Larson, 1985).

The results of the first experiment indicated that cockroaches responded to ultrasound; however, the practical importance of these observations is low as a significant number of cockroaches did not move from the chamber where the ultrasound device was used. Ballard et al. (1984)

reported that the use of ultrasound in laboratory experiments caused an increase in movement in a confined German cockroach population. However, statistical separation of mean cockroach numbers on a daily basis suggested habituation on days 6 and 7. These results provide additional evidence that ultrasound has marginal ability in repelling cockroaches and the repellency is not practically important in terms of managing cockroach populations.

In conclusion, our studies with a random ultrasound generating device using higher sound frequencies than those reported previously by researchers, confirmed ultrasound to be ineffective in repelling mosquitoes and German cockroaches. However, future studies using ultrasound as an effective means of controlling pest behavior is still open. For example, an approach directed to male attraction to ultrasound (high to low frequency) could be useful in a population control strategy or mating disruption (Ikeshoji et al., 1985; Belton, 1994) but not for repelling female mosquitoes. Likewise, the possibility of repelling cockroaches using a combination of ultrasound and light should be explored.

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